

CLAIMS

1. A spheroid tissue microarray comprising an array of tissue spheroids embedded within a porous mold, in which the spheroids are horizontally arranged substantially in a regular square array such that the centrepoint of each spheroid is no more than 100 micrometers from its
5 desired ideal horizontal location.
2. A spheroid tissue microarray according to Claim 1 in which the porous mold is formed from an agarose or agar gel and/or in which the spheroid tissue microarray is impregnated with an additional structural agent, which optionally is paraffin wax or a resin.
3. A spheroid tissue microarray according to Claim 1 or 2 which is formed to contain a
10 series of regularly positioned holes arranged in a grid pattern:
 - in which the holes are between 0.2 and 3.0mm diameter, optionally are between 0.5 and 1.5mm diameter, or optionally are approximately 1mm diameter; and/or
 - in which the holes have a depth of between 2 and 5mm, optionally have a depth of between 3 and 4mm, or optionally have a depth of approximately 4mm; and/or
 - 15 in which the porous mold contains from 100 to 300 holes, optionally comprises a 10 x 17 grid of 170 holes, or in which the porous mold contains 96 holes.
4. A spheroid tissue microarray according to Claim 1, 2 or 3 in which the density of spheroids per cm² is between 100 and 500.
5. A spheroid tissue microarray according to any preceding claim in which the tissue
20 spheroids comprise tissue derived from one or more of induced pluripotent stem cells, primary cells, cell lines derived from cancerous tissues, neuronal cells, skin cells, liver cells, tumour cells, stromal cells, immune cells, and endothelial cells.
6. A spheroid tissue microarray according to any preceding claim in which the tissue spheroids are formed from the *in vitro* 3D culture of cells.
- 25 7. A spheroid tissue microarray according to any preceding claim in which the tissue spheroids have a diameter of 50 to 500µm, optionally a diameter of 100 to 500µm, or optionally a diameter of 150µm or/to 350µm.

8. A spheroid tissue microarray comprising an array of tissue spheroids embedded within a porous mold in which the tissue spheroids comprise a substrate on which cells are grown prior to embedding, optionally in which the substrate is a bead formed from polystyrene (amberlite) or another porous plastic material, and/or optionally in which the tissue spheroids comprise human skin cells.
9. A spheroid tissue microarray according to any preceding claim in which the tissue spheroids are stained for histological analysis.
10. A method of manufacturing a spheroid tissue microarray, which method comprises the steps of:
- 10 forming a mold of porous material from liquid mold material in a casting mold, and allowing the liquid mold material to set;
- removing the porous mold from the casting mold;
- topping up the porous mold with further liquid mold material, and allowing recesses to form in the surface of the mold by the drawing-in of liquid mold material through shrinkage as the liquid mold material sets;
- 15 placing tissue spheroids into the recesses in the surface of the porous mold; and sealing the tissue spheroids within the mold by topping off with liquid mold material and allowing the liquid mold material to set.
11. A method according to Claim 10 for manufacturing a spheroid tissue microarray according to any of Claim 1 to 9.
- 20 12. A method according to Claim 10 or 11:
- in which the liquid mold material is 0.5 to 4% agarose in water, optionally a 2% agarose/water mixture; and/or
- in which the step of forming the mold of porous material from liquid mold material in a casting mold is performed at a temperature of from 60 to 70°C; and/or
- 25 in which the casting mold is pre-equilibrated to a suitable temperature, optionally 70°C; and/or

- in which the liquid mold material is allowed to set in the casting mold at room temperature for 20 to 45 minutes, followed by chilling at 0 to 5°C for 20 to 45 minutes, optionally in which the liquid mold material is allowed to set at room temperature for approximately 30 minutes, followed by chilling at approximately 4°C for approximately 30 minutes; and/or
- 5 in which the porous mold is stiffened for removal from the casting mold by chilling at a temperature of approximately -20°C for 5 to 15 minutes; and/or
- in which prior to topping up, the porous mold is heated at 60 to 70°C for 10 to 30 minutes in a sealed environment; and/or
- 10 in which the porous mold is topped up with liquid mold material comprising an agarose gel at 0.1 to 1.0% in water, optionally 0.7% in water; and/or
- in which a suitable dye marker is added to the topping-up liquid mold material, optionally in which the dye marker is bromophenol blue; and/or
- in which the recesses are formed within the top 1mm of the mold; and/or
- 15 in which the tissue spheroids are sealed within the mold recesses by topping off with liquid mold material at a temperature of from 60 to 70°C; and/or
- in which following sealing of the tissue spheroids within the mold recesses, the liquid mold material is allowed to set by cooling to 0 to 5°C for approximately 30 minutes; and/or
- 20 in which the spheroid tissue microarray is impregnated with an additional structural agent, optionally paraffin wax or a resin, optionally in which the paraffin wax is impregnated into the spheroid tissue array in cycles of approximately two hours at 60°C, under atmospheric pressure or lower pressure.
13. A method of manufacturing a spheroid tissue microarray, which method comprises the
- 25 steps of: forming a mold of porous material from liquid mold material in a casting mold; allowing the liquid mold material to set; removing the porous mold from the casting mold; placing spheroids in recesses at the bases of wells in the mold of porous material; and sealing the spheroids within the porous mold by adding further porous material on top of the spheroids; in which the recesses at the bases of the wells in the porous material are formed by
- 30 protrusions of the casting mold carrying further, nipple-shaped, protrusions.

14. A method according to Claim 13 further comprising a subsequent step of impregnating the microarray with an additional structural agent, optionally in which the structural agent is paraffin wax or a resin.
15. Use of a spheroid tissue microarray according to any of Claims 1 to 9 in an analytical
5 method comprising sectioning the spheroid tissue microarray so as to simultaneously section multiple spheroids and analysing the sections.